sudden release of pressure. The method further comprises heat treating the microfluidized lysate preparation. The *Leishmania* parasite may be *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum,* or *L. aethiopica.*

[13] In some embodiments, the present invention relates to a microfluidized lysate preparation made by microfluidizing a slurry of at least one *Leishmania* parasite through a chamber and disrupting the leishmania parasite with a sudden release of pressure and heat treating the microfluidized lysate preparation. The *Leishmania* parasite may be *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum,* or *L. aethiopica.*

[14] In some embodiments, the present invention relates to a skin test antigen assay for detecting whether a subject has been exposed to a *Leishmania* parasite or was afflicted with Leishmaniasis comprising administering to the subject an antigenic amount of at least one microfluidized lysate preparation of the present invention and observing any immunogenic response to the microfluidized lysate preparation. The *Leishmania* parasite may be *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum,* or *L. aethiopica.* An immunogenic response indicates that the subject has been exposed to a *Leishmania* parasite or was afflicted with Leishmaniasis. Preferably, an induration of about 5 mm or greater observed indicates that the subject has been exposed to a *Leishmania* parasite or was afflicted with Leishmaniasis. In preferred embodiments, the antigenic amount of the microfluidized lysate preparation comprises about 5 μg to about 30 μg of total protein. The microfluidized lysate preparation is administered intradermally to the volar surface of the forearm of the subject.

[15] In some embodiments, the present invention relates to a kit comprising the microfluidized lysate preparation of the present invention and directions for determining whether a subject has been exposed to a *Leishmania* parasite or was afflicted with Leishmaniasis. The *Leishmania* parasite may be *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum,* or *L. aethiopica.* The kit may further comprise at least one pharmaceutical for treating systemic anaphylaxis such as epinephrine, diphenhydramine, and methyl prednisolone. The kit may further comprise at least one

pharmaceutical for treating local reactions to the microfluidized lysate preparation such as hydrocortisone, hydrocortisone cream, acetaminophen, or diphenhydramine.

[16] In some embodiments, the present invention relates to antibodies raised against the microfluidized lysate preparation of the present invention.

[17] In some embodiments, the present invention relates to a method of determining whether a subject has been exposed to a given *Leishmania* parasite comprising administering to the subject a panel of antigenic compositions comprising a plurality of microfluidized lysate preparations prepared from a plurality of *Leishmania* parasites and detecting a presence of an immunogenic reaction that is characteristic to exposure to the given *Leishmania* parasite. The plurality of *Leishmania* parasites may include at least one parasite belonging to the group consisting of *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum,* and *L. aethiopica.*

[18] In some embodiments, the present invention relates to a method of immunizing a subject against Leishmaniasis comprising administering to the subject an immunogenic amount of the microfluidized lysate preparation.

[19] In some embodiments, the present invention relates to a pharmaceutical composition comprising the microfluidized lysate preparation and a pharmaceutically acceptable stabilizer such as phenol. In preferred embodiments, the pharmaceutical composition is in the form of a liquid which may be frozen or freeze-dried.

[20] In some embodiments, the present invention relates to a method for determining post infection of cutaneous leishmaniasis, mucocutaneous leishmaniasis, or post-kala-azar dermal leishmaniasis in a subject comprising administering to the subject an antigenic amount of at least one microfluidized lysate preparation and observing any immunogenic response to the microfluidized lysate preparation.

[21] In some embodiments, the present invention relates to a method for epidemiologically diagnosing cutaneous leishmaniasis, mucocutaneous leishmaniasis, or post-kala-azar dermal leishmaniasis in a subject comprising administering to the subject an antigenic amount of at least one microfluidized lysate preparation and observing any immunogenic response to the microfluidized lysate preparation.

[22] In some embodiments, the present invention relates to a method for determining the pattern of present and past leishmaniasis in a subject comprising administering to the

subject an antigenic amount of at least one microfluidized lysate preparation and observing any immunogenic response to the microfluidized lysate preparation.

[23] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed. The accompanying drawings are included to provide a further understanding of the invention and are incorporated in and constitute part of this specification, illustrate several embodiments of the invention and together with the description serve to explain the principles of the invention.

## DESCRIPTION OF THE DRAWINGS

[24] This invention is further understood by reference to the drawings wherein:

[25] Figure 1 is a flow diagram showing the process for making a Leishmaniasis microfluidized lysate according to the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

[26] The present invention allows the detection of post infections and epidemiological diagnosis of all forms of leishmaniasis. The present invention may be used for screening of individuals such as U.S. Service Members who may have been exposed to Leishmania parasites after deployment to Leishmaniasis endemic areas. The present invention may be used in the clinical diagnosis of cutaneous leishmaniasis, mucocutaneous leishmaniasis, and post-kala-azar dermal leishmaniasis in subjects such as U.S. Service Members. The present invention may be used to determine the pattern of past and present infections of leishmaniasis.

[27] The present invention provides microfluidized lysate preparations comprising at least one antigen from at least one *Leishmania* parasite and methods of making and using thereof. The microfluidized lysate preparations of the present invention may be used to elicit induration that is consistent with delayed type hypersensitivity (DTH) in an individual previously infected with or exposed to at least one *Leishmania* parasite. Preferably, the microfluidized lysate preparation is injected intradermally. Thus, the present invention also provides a skin test assay for detecting whether a subject has been exposed to a *Leishmania* parasite or is or has been afflicted with Leishmaniasis comprising at least one microfluidized lysate preparation of at least one *Leishmania* parasite.